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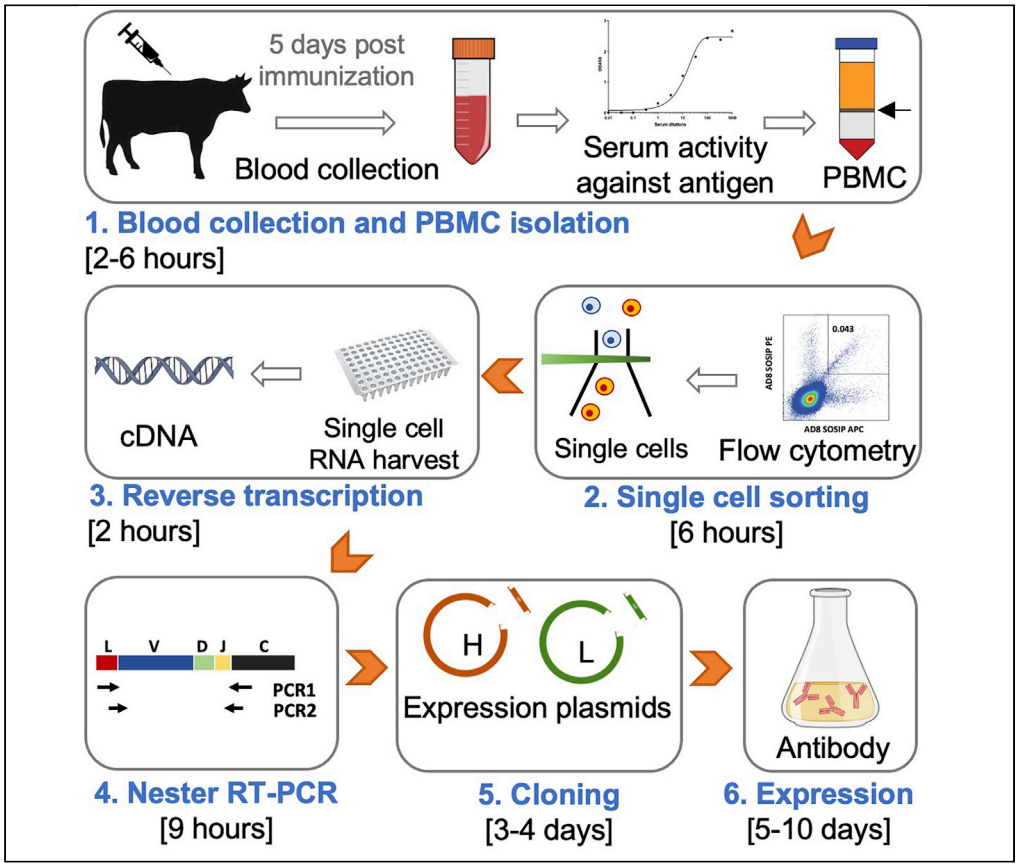
Protocol for production and expression of chimeric bovine-human monoclonal antibodies



We describe herein a protocol for production of chimeric bovine-human monoclonal antibodies (mAbs) from vaccinated cows. The genes of HIV-1-specific single B cells are amplified by reverse transcription-polymerase chain reaction (RT-PCR), cloned into human expression vectors, and expressed in human cell lines. This protocol provides an efficient step-by-step methodology to produce HIV-1 chimeric mAbs and could be widely adapted for other antigens.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Sorting of bovine HIV-1-specific B cells into 96-well plates

cDNA synthesis and amplification of bovine variable genes from single B cells

Cloning of bovine heavy and light variable genes into human expression vectors

Expression of chimeric bovine-human monoclonal antibodies in the human Expi293 cell line

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Protocol for production and expression of chimeric bovine-human monoclonal antibodies

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SUMMARY

We describe herein a protocol for production of chimeric bovine-human monoclonal antibodies (mAbs) from vaccinated cows. The genes of HIV-1-specific single B cells are amplified by reverse transcription-polymerase chain reaction (RT-PCR), cloned into human expression vectors, and expressed in human cell lines. This protocol provides an efficient step-by-step methodology to produce HIV-1 chimeric mAbs and could be widely adapted for other antigens.
For complete details on the use and execution of this protocol, please refer to Heydarchi et al. (2022).[1]

BEFORE YOU BEGIN

This protocol describes how to produce antigen-specific chimeric bovine-human mAbs with the following steps: Isolation of antigen-specific B cells, sequencing and cloning of bovine antibody variable genes and expression of chimeric bovine-human mAbs. The current protocol is an adaptation of the methods described previously[1–3] which resulted in isolation and production of ultra-potent cross-clade neutralizing chimeric bovine-human mAbs.

Institutional permissions

All bovine experiments will need to comply with protocols approved by a local animal ethics committee (This work was conducted under animal ethics approval 2015–17 from the Victorian State Government DEDJTR Research and Extension Animal Ethics Committee).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse Anti-Bovine IgG Monoclonal Antibody, Unconjugated, Clone BG-18 (working dilution: 1 in 200)	Sigma-Aldrich	Cat# B6901, RRID: AB_258594
D7324 Sheep anti-gp120 (working concentration: 2 μg/mL)	Aalto Bio Reagents	Cat# D7324 (Used for production of anti-HIV chimeric antibody)
Goat anti-human IgG HRP (working dilution: 1 in 1000)	KPL	Cat# 474-1002
Biological samples		
Bovine PBMC samples	Heydarchi et al.[1]	N/A

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Streptavidin-allophycocyanin (APC)	Thermo Fisher Scientific	Cat# S868
Streptavidin-phycoerythrin (PE)	Thermo Fisher Scientific	Cat# S866
DPBS, pH 7.4	Thermo Fisher Scientific	Cat# 14190144
Horse serum	Sigma-Aldrich	Cat# H1138
RPMI 1640 medium	Thermo Fisher Scientific	Cat# 11875093
NucleoSpin Gel and PCR Clean-up	Macherey-Nagel	Cat# 740609.250
NucleoBond Xtra Midi	Macherey-Nagel	Cat# 740410.10
NucleoSpin Plasmid	Macherey-Nagel	Cat# 740499.250
Ficol paque™ plus	GE Healthcare	Cat# Cytiva 17-1440-02
SureBlue™ TMB 1-Component Microwell Peroxidase Substrate	KPL	Cat# 5120-0075
D7324 tagged- AD8 SOSIP gp140. V4.1	Heydarchi et al.[1]	N/A (Used for production of anti-HIV bovine antibody)
Biotinylated AD8 SOSIP gp140. V4.1	Heydarchi et al.[1]	N/A (Used for production of anti-HIV chimeric antibody)
Critical commercial assays		
BirA Biotin-Protein Ligase Kit	Avidity	BirA-500
RQ1 RNase-Free DNase	Promega	Cat# M6101
LIVE/DEAD™ Fixable Aqua Dead Cell Stain	Thermo Fisher Scientific	Cat# L34965
RNasin Plus Ribonuclease Inhibitor	Promega	Cat #N2615
SuperScript™ III First-Strand Synthesis System	Thermo Fisher Scientific	Cat# 18080051
Promega Ultra Pure Deoxynucleotide Triphosphates (dNTPs) Sets	Promega	Cat# U1330
MyTaq HS Red Mix	Bioline	Cat# BIO-25048
EcoRI-HF Restriction enzyme	New England Biolabs	Cat# R3101L
NheI-HF Restriction enzyme	New England Biolabs	Cat# R3131LL
AvrII Restriction enzyme	New England Biolabs	Cat# R0174L
T4 DNA ligase	Thermo Fisher Scientific	Cat# EL0012
ExpiFectamine™ 293 Transfection Kit	Thermo Fisher Scientific	Cat# A14524
Protein G Agarose Fast Flow	Millipore	Cat# A16266
RNaseZap	Thermo Fisher Scientific	Cat# AM9780
Ficoll-Paque	GE Healthcare	Cat# 17-1440-02
Experimental models: Cell lines		
Expi293F cells	Thermo Fisher Scientific Inc	Cat# A14525
Oligonucleotides		
Odp 2569 (forward): ATGAACCCACTGTGGACCCTC	Heydarchi et al.[1]	N/A
Odp 2571 (Forward-*EcoRI*): GAATTCGCAGGCTGTGCTGACTCAG	Heydarchi et al.[1]	N/A
Odp 2573 (Forward): ATGTCCACCATGGCCTGGTCC	Heydarchi et al.[1]	N/A
Odp 3668 (Forward- *EcoRI*): GAATTCGMAGGTGCAGCTGCRGGAGTC	Heydarchi et al.[1]	N/A
Odp 3670 (Forward): CACCATGGCCTGGTCCCCTCTG	Heydarchi et al.[1]	N/A
Odp 3671 (Forward): GACCCCAGACTCACCATCTC	Heydarchi et al.[1]	N/A
Odp 3672 (Forward): AGGGCTGCGGGCTCAGAAGGCAGC	Heydarchi et al.[1]	N/A
Odp 3673 (Forward): CTGCCCCTCCTCACTCTCTGC	Heydarchi et al.[1]	N/A
Odp 3674 (Forward): GGAACCTTTCCTGCAGCTC	Heydarchi et al.[1]	N/A
Odp 3675 (Forward): GCTTGCTTATGGCTCAGGTC	Heydarchi et al.[1]	N/A
Odp 2568 (Reverse-*NheI*): GCTAGCTGAGGAGACGGTGACCAGGAG	Heydarchi et al.[1]	N/A
Odp 2570: (Reverse) AGAACTCAGAGGGTAGACTTTCGG	Heydarchi et al.[1]	N/A
Odp 2574 (Reverse): CTTGTTGCCGTTGAGCTCCTC	Heydarchi et al.[1]	N/A
Odp 3667 (Reverse): CTTTCGGGGCTGTGGTGGAGGC	Heydarchi et al.[1]	N/A
Odp 3677 (Reverse- *AvrII*): CCTAGGACGACKGTCAGTGTGGTSCC	Heydarchi et al.[1]	N/A
Odp 2781 sequencing: CTCAACTCTACGTCTTTGTTTC	Heydarchi et al.[1]	N/A
Recombinant DNA		
pFUSE2ss-CLIg-hL2	InvivoGen	Cat#pfuse2ss-hcll2
pFUSEssCHIg-hG1	InvivoGen	Cat# pfusess-hchg1

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
IgBlast	(Ye et al.[4])	http://www.ncbi.nlm.nih.gov/igblast/
IMGT/V-QUEST	(Brochet et al.,[5] Giudicelli et al.[6])	http://www.imgt.org/IMGT_vquest/vquest
GraphPad Prism Software	GraphPad Prism Software, Inc.	SCR_002798
FlowJo software v.10	BD biosciences	SCR_008520
ImageJ	Schneider et al.)[7]	https://imagej.nih.gov/ij/
Other		
96 Well Semi-Skirted PCR Plate	INTEGRATED SCIENCES	Cat# GENIUS760
F96 Maxisorp flat bottom plates	Nunc	Cat# 442404
Amicon Centrifugal Filter	Merck	Cat# UFC905024; # UFC805024
Adhesive PCR Plate Seals	INTEGRATED SCIENCES	Cat# GENIUS500
BD FACSAria III cell sorter	BD	N/A
PCR Thermal Cycler	Eppendorf	MasterCycler Nexus

STEP-BY-STEP METHOD DETAILS

Processing and storage of bovine PBMC

Timing: 2–6 h (for steps 1 to 16)

This step describes a workflow for processing and storage of bovine PBMC required for isolation of antigen specific B cells.

1. Collect blood (50–100 mL) from jugular vein of the cow using a butterfly cannula into blood bag containing citrate phosphate dextrose anticoagulant. Ship the blood samples at 20°C–25°C on the same-day or for 12–18 h courier to the processing lab.

   Note: We were successful using blood draws made five days after a booster vaccination when memory B-cells are likely to be in peripheral circulation.

   Note: Simultaneously blood without anticoagulants need to be collected to confirm the reactivity of serum against vaccine immunogen.

2. Dilute blood with 20°C–25°C DPBS-2 mM EDTA (1:1 v/v).
3. Add 20 mL Ficoll-Paque to an empty 50 mL conical centrifuge tube.
4. Carefully layer 30 mL of diluted blood onto Ficoll-Paque.

   Note: Do not mix the blood and Ficoll- Paque media.

   Note: Seven 50 mL conical centrifuge tube containing Ficoll-Paque (GE-Healthcare) is required if collecting 100 mL blood.

5. Centrifuge at 800 × *g* for 20 min at 20°C–25°C (brake: off; acceleration: slow).

   Note: Set the centrifuge at 4°C after this step.

6. Using a sterile pipette, carefully discard the upper layer containing plasma and platelets. Do not disturb the white mononuclear cell layer.

   Note: Diluted plasma in the upper layer can be stored and tested to determine the titre of antibodies against the target antigen. Antibodies can also be purified from diluted plasma if required.

7. Use a transfer pipette in a slow, circular motion to carefully suck up mononuclear cells (the white layer like a white cloud on top of Ficoll-Paque layer). Transfer the cells into an empty sterile 50 mL conical centrifuge tube.
8. Add at least 2 volumes of cold DPBS-2 mM EDTA (4°C–8°C) to the transferred mononuclear cells.
9. Resuspend the cells gently and horizontally centrifuge at 325 × *g* for 8 min at 4°C (brake: off; acceleration: slow).

   Note: If it is important to get rid of platelets, centrifuge the cells at low speed (100–200 × *g*) for 10 min at 4°C.

10. Discard the supernatant and resuspend the mononuclear cells in 2 mL cold DPBS-2 mM EDTA and pool the cells from 50 mL conical centrifuge tubes into one 50 mL centrifuge tube. Fill to top with cold DPBS-2 mM EDTA.
11. Horizontally centrifuge at 150 × *g* for 8 min at 4°C (brake: off; acceleration: slow). Discard the supernatant.
12. Resuspend the cells in 3–4 mL 0.83% ammonium chloride and incubate for 5 min on ice.
13. Wash the cells with 20 mL cold DPBS-2 mM EDTA and centrifuge at 150 × *g* for 8 min at 4°C (brake: on; acceleration: high).
14. Discard the supernatant and repeat the wash step two more times. Resuspend the cells in cold DPBS-2 mM EDTA and count the cells.
15. Centrifuge at 150 × *g* for 8 min at 4°C (brake: on; acceleration: high).
16. Discard the supernatant and resuspend the cells in freezing media containing 90% horse serum (Sigma Aldrich) and 10% dimethylsulfoxide (DMSO).

    Note: Freeze 10–20 million cells per 1 mL freezing media.

    Note: Label the microtubes before aliquoting the cells as it is not recommended to keep the cell in cryopreservation medium at 20°C–25°C. Place the cryovials in the freezing container and store them at −80°C for 24 h (for long-term storage, transfer the vials to liquid nitrogen).

Staining and sorting of antigen-specific B cells

Timing: ~ 6 h (for steps 17 to 43)

The protocol for staining and sorting of cells needs to be designed and tested in advance according to the user's experimental needs. To clarify the strategy, we describe using a panel of antibodies to sort HIV-1 envelope (Env)-specific B cells in this step.

Biotinylation of HIV-1 Env probe

17. Add 0.15 nmol of Avi-tagged HIV-1 AD8 SOSIP v4.1 Env (130 kDa; 20 µL from 1 mg/mL stock) to one sterile 1.5 mL microtube.

    Note: AD8 SOSIP is a native like stabilized cleavable version of Env.[1] Avi-tag is an extra 15 amino acid sequence that can be fused to the N- or the C-terminus of protein (C-terminus in this study). BirA ligase enzymatically attaches one biotin molecule to Avi-tag.

    ⚠ CRITICAL: HIV-1 Env need to be in Bicine 50 mM buffer for efficient biotinylation. Change the buffer before this step if it is required.

18. Add 0.5 µg (1.19 µL from 0.42 mg/mL stock) of BirA enzyme from BirA Biotin-Protein Ligase Kit (Avidity) to microtube.

Note: 2.5 μg of BirA enzyme is required per 10 n mol protein in 40 μM final volume).

19. Add 3 μL Biomix A and 3 μL Biomix B from BirA Biotin-Protein Ligase Kit (Avidity) to the microtube.
20. Adjust the final reaction volume to 30 μL with ddH_2O (2.81 μL).
21. Incubate the reaction at 30°C for 40 min.
22. Change the buffer to DPBS using 50 kDa Amicon Ultra centrifugal filter units according to the manufacturer's instruction.

Note: Adjust the final volume to 20–100 μL (final concentration 0.2–1 mg/mL).

⚠ CRITICAL: Efficient biotinylation and retained antigen activity must be confirmed according to the user's experimental needs before fluorescent labelling. Biotinylation can be checked in ELISA assay by coating the wells with biotinylated Env then detecting the biotin with streptavidin-HRP. Antigenicity and the presence of epitopes can be tested using different antibodies.

Fluorescent labelling of sorting probe

Couple HIV-1 Env to streptavidin-APC and streptavidin-PE (Thermo Fisher Scientific) separately in equimolar ratios as following:

Note: If double staining is not required, coupling can be limited to APC as a very bright fluorochrome with minimal spectral overlap with Alexa-488-IgG.

23. Add 5.54 μL Streptavidin-PE to sterile 1.5 mL microtube containing 6.46 μL DPBS (Gibco). Mix it well and cover the microtube with aluminium foil to protect from light. Keep the microtube on ice.
24. Add 4.10 μL Streptavidin-APC to another sterile 1.5 mL microtube containing 7.90 μL DPBS (Gibco). Mix it well and cover the microtube with aluminium foil to protect from light. Keep the microtube on ice.
25. In two separate microtube, add 8 μg (1 mg/mL) HIV-1 Env AD8 SOSIP v4.1 and label them as PE and APC, respectively.
26. Add 2.4 μL Streptavidin-PE/DPBS mixture to microtube PE, cover the microtube with aluminium foil to protect from light and incubate for 15 min on ice while shaking (or use a rotary shaker at 4°C).
27. Repeat step 26, four more time (each time 2.4 μL) to add all Streptavidin-PE/DPBS mixture to HIV-1 Env probe.

Note: Keep the microtube at 4°C until use for maximum 24 h.

28. Repeat step 22 for APC microtube using Streptavidin-APC/DPBS mixture.

Staining and fluorescence-activated cell (FACS) sorting of antigen-specific bovine B cells

This step can be performed by both cryopreserved or freshly isolated PBMC. If using cryopreserved PBMCs, start with step 29 and if using freshly isolated PBMCs, start with step 37.

29. Thaw 2–3 million cryopreserved PBMCs in 37°C water batch until there is just a small bit of ice left in the vial.
30. Add 500 μL pre-warmed 37°C RPMI 1640 medium (Life technologies) (containing 10% FBS, 20 μg/mL or 10 U/mL RQ1 RNase -free DNaseI (Promega)) dropwise. Resuspend the cells.
31. Transfer the cells to one sterile 10 mL conical centrifuge tube and add 9.5 mL pre-warmed RPMI (as above) slowly. Incubate for 5 min at RT.
32. Filter the cells through a 40-μm cell strainer to remove clumps followed by horizontal centrifugation at 200 × *g* for 10 min at 4°C.

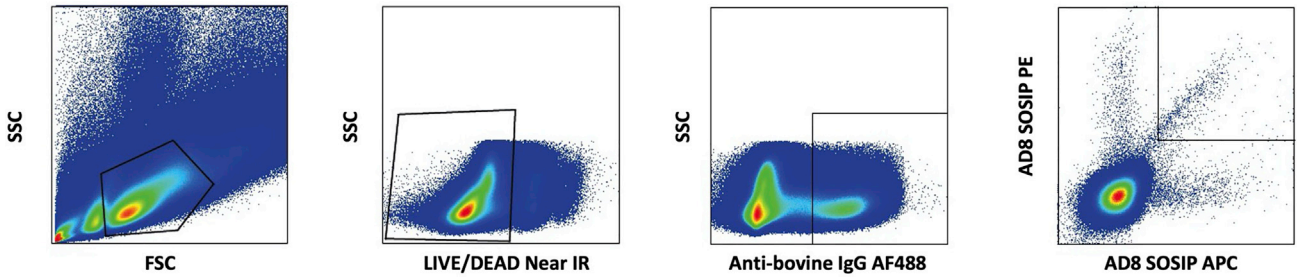

Figure 1. FACS sorting gating strategy of HIV-1 specific bovine B cells

Cow PBMCs are sorted for live IgG+ cells that bind to biotinylated AD8 SOSIP-AviTag conjugated to PE and APC fluorophores.

33. Discard the supernatant and resuspend the cells in 6 mL cold DPBS-2 mM EDTA-1% horse serum.
34. Add 4 mL Ficoll-Paque to one sterile 10 mL conical centrifuge tube. Layer the cells onto the Ficoll-Paque and centrifuge at 500 × *g* for 20 min at 4°C (brake: off; acceleration: slow).

⚠ CRITICAL: This step removes the dead cells which improves the efficiency of staining.

35. Collect the white layer on top of Ficoll-Paque and transfer to one sterile 10 mL conical centrifuge tube. Add 9 mL cold DPBS-2 mM EDTA-1% horse serum.
36. Centrifuge at 500 × *g* for 10 min at 4°C (brake: on; acceleration: high) and discard the supernatant.
37. Resuspend the cells in 157.9 µL cold DPBS-2 mM EDTA and transfer them to one sterile 5 mL FACS tube.
38. Add 0.1 µL LIVE/DEAD™ Fixable Aqua Dead Cell Stain (Thermo Fisher Scientific) and incubate the cells on ice for 10–15 min.
39. Add 20 µL HIV-1 Env-PE, 20 µL HIV-1 Env-APC and 2 µL Alexa-fluor 488 conjugated anti-bovine IgG (Sigma, B6901). Incubate the cells for 1 h on ice (on a rocking mixer).

Note: anti-bovine IgG (clone B6901) is in unconjugated format and need to be conjugated to Alexa-fluor 488 before the staining.

40. Add 4 mL DPBS containing 1 mM EDTA and 1% horse serum (Sigma) and centrifuge at 500 × *g* for 10 min.
41. Resuspend the cells in 200–500 µL DPBS-2 mM EDTA-1% horse serum and filter through a 40-µm cell strainer to remove clumps.
42. Gate (Figure 1) and sort single cells (live/ IgG+/ HIV Env-PE+/HIV Env-APC+) into 96-well plates containing lysis buffer (3.7 µL/well DPBS, 10 mM DTT and 8 U RNasin Ribonuclease Inhibitor (Promega)) on an ARIA III sorter using BD FACSDiva Software.

Note: Frequency of IgG+ cells can be 5%–20% of live cells.

43. Once the sorting is finalised, cover the plate with adhesive PCR plate seals, centrifuge it at 1,000 × *g* for 30 s and store the plate at −80°C immediately.

Pause point: The plate is stable at −80°C for a few months. If the plate is immediately flash frozen on dry ice, it can be stored at −80°C for even several years.

⚠ CRITICAL: The plate can't be proceeded to next step without the cold shock or storage at −80°C. This step is required to rupture the cell membranes.

Reverse transcription and nested RT-PCR

Timing: ~ 11 h (for steps 44 to 62)

This step describes the steps required for cDNA synthesis from bovine single B cells and amplification of variable genes in nested RT-PCR.

⚠ CRITICAL: Decontaminate the hood, bench surface, pipettes prior to use with RNaseZap (Thermo Fisher Scientific) to avoid any possible contamination. Use RNase-free precautions for this step.

cDNA synthesis

44. Thaw the plate of single cells on ice for 5 min.
45. Prepare the reverse transcription master mix on ice.

Note: Prepare dNTP (10 mM final concentration for each deoxynucleoside triphosphate) by mixing stock of dATP, dTTP, dCTP, and dGTP solutions at 1:1:1:1 (Promega) ratio. Aliquot and store them at −20°C. Alternatively, can use premixed dNTP available from Invitrogen or other providers.

Reverse transcription master mix

Reagent	Final concentration in reverse transcription reaction	Amount
Random Hexamer	5–10 ng/ µL	1 µL (100–200 ng)
dNTP (10 mM)	0.5 mM	1 µL
DTT 0.1 M (supplied with SuperScript™ III Reverse Transcriptase)	5 mM	1 µL
RNasin® Ribonuclease Inhibitors (Promega, #N2115)	0.4 U/ µL	0.2 µL (8 U)
SuperScript™ III First-Strand Synthesis System (ThermoFisher, #18080051)	2.5 U/ µL	0.25 µL (40 U)
Buffer 5× supplied with SuperScript™ III Reverse Transcriptase)	1×	4 µL
Nuclease-free H_2O	NA	7.55 µL
Total	**NA**	**15 µL**

46. Carefully add 15 µL of the master mix to each well, mix.
47. Cover the plate with adhesive PCR plate seals.
48. Centrifuge the plate 1,000 × *g* for 30 s and use the reverse transcription program.

Reverse transcription cycling conditions

Temperature	Time	Cycles
42°C	10 min	1
25°C	10 min	1
50°C	60 min	1
94°C	5 min	1
4°C	Forever	

Pause point: Store cDNA plate at −20°C or −80°C (stable for months).

Note: Plate can be proceeded to the next step immediately (without any need to be stored at −20°C).

PCR1

49. Prepare PCR1 master mix.

Note: add 0.25 μL from each primer to the master mix.

50. Label a plate as PCR1 and add 20 μL of PCR 1 master mix to each well.

Note: heavy and light chain reactions must be prepared and performed in separate plates.

51. Thaw cDNA plate on ice for 5 min then centrifuge at 1,000 × *g* for 30 s.
52. Add 5 μL cDNA (one fourth of reverse transcription product) to PCR1 plate.

Note: one tenth to one fourth of reverse transcription product can be used in PCR1.

53. Cover the plate with adhesive PCR plate seals.
54. Centrifuge the plate 1,000 × *g* for 30 s place the plate in thermocycler and run PCR1 program.

PCR1 master mix

Reagent	Final concentration in PCR1 reaction	Amount
MyTaq HS Red Mix (Bioline, # BIO-25046)	2×	12.5 μL
Forward primer (each primer) 20 μM	250 mM (each primer)	0.25 μL
Reverse Primer (each primer) 20 μM	250 mM (each primer)	0.25 μL
Nuclease-free H_2O	NA	Up to 20 μL
Total	**NA**	**20 μL**

PCR1 cycling conditions

Steps	Temperature	Time	Cycles
Initial denaturation	94°C	5 min	1
Denaturation	94°C	45 s	50 cycles
Annealing	60°C or 58°C *	45 s	
Extension	72°C	45 s	
Final extension	72°C	10 min	1
Hold	4°C	Forever	

* Annealing temperature for heavy gene PCR1 reaction is 60°C while for the light gene PCR1 is 58°C.

Pause point: Remove the PCR1 plate from the thermocycler and store it at −20°C or −80°C (stable for months).

Note: Plate can be proceeded to the next step immediately (without any need to be stored at −20°C).

Note: Primers for PCR1 of antibody heavy variable genes (Figure 2):

Odp 2569 (forward): ATGAACCCACTGTGGACCCTC.

Odp 2570: (Reverse) AGAACTCAGAGGGTAGACTTTCGG.

Odp 3667 (Reverse): CTTTCGGGGCTGTGGTGGAGGC.

Note: Primers for PCR1 of antibody light variable genes (Figure 2):

Odp 3670 (Forward): CACCATGGCCTGGTCCCCTCTG.

Odp 3671 (Forward): GACCCCAGACTCACCATCTC.

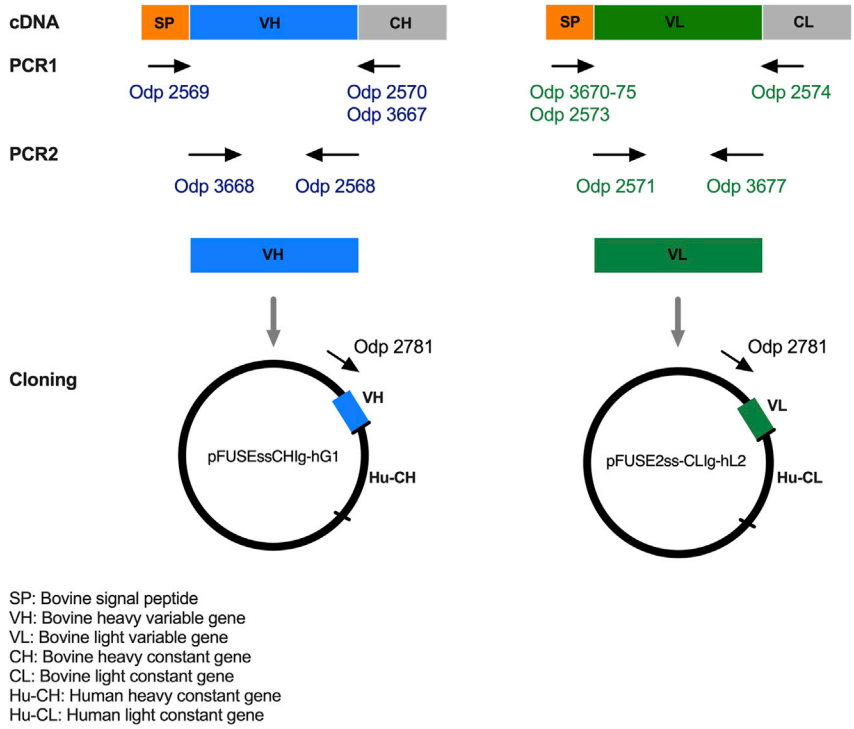

Figure 2. Strategy to construct chimeric bovin-human mAb

Bovine heavy and light mAb variable genes are amplified in nested RT-PCR (including PCR1 and PCR2). Then, the genes are cloned into the expression vectors containing human heavy and light antibody constant genes.

Odp 3672 (Forward): AGGGCTGCGGGCTCAGAAGGCAGC.

Odp 3673 (Forward): CTGCCCCTCCTCACTCTCTGC.

Odp 3674 (Forward): GGAACCTTTCCTGCAGCTC.

Odp 3675 (Forward): GCTTGCTTATGGCTCAGGTC.

Odp 2573 (Forward): ATGTCCACCATGGCCTGGTCC.

Odp 2574 (Reverse): CTTGTTGCCGTTGAGCTCCTC.

PCR2

55. Prepare PCR2 master mix.

Note: add 0.5 μL from each primer to the master mix.

56. Label a plate as PCR2 and add 45 μL of PCR2 master mix to each well and keep the plate on ice.

Note: heavy and light chain reactions must be prepared and performed in separate plates.

57. Thaw PCR1 plate on ice for 5 min then centrifuge at 1,000 × *g* for 30 s and place it on ice again.
58. Add 5 μL of PCR1 product to PCR2 plate.

Note: Cover PCR1 plate and store the remaining PCR product at −20°C.

59. Centrifuge PCR2 plate at 1,000 × *g* for 30 s, place the plate in the thermocycler and run PCR2 program.

PCR2 master mix		
Reagent	Final concentration in PCR2 reaction	Amount
MyTaq HS Red Mix (Bioline, # BIO-25046)	2×	25 μL
Forward primer (each primer)	250 mM (each primer)	0.5 μL
Reverse Primer (each primer)	250 mM (each primer)	0.5 μL
Nuclease-free H_2O	NA	Up to 45 μL
Total	**NA**	**45 μL**

PCR2 cycling conditions			
Steps	Temperature	Time	Cycles
Initial denaturation	94°C	5 min	1
Denaturation	94°C	45 s	50 cycles
Annealing	60°C	45 s	
Extension	72°C	45 s	
Final extension	72°C	10 min	1
Hold	4°C	Forever	

Note: Primers for PCR2 of antibody heavy variable genes (Figure 2) (restriction enzyme sites are shown with underline). To increase the cleavage efficiency, several base pairs (NNNNN) should be added on either side of the recognition site of restricted enzymes.

Odp 3668 (Forward- *EcoRI*): NNNNNGAATTCGMAGGTGCAGCTGCRGGAGTC.

Odp 2568 (Reverse-*NheI*): NNNNNGCTAGCTGAGGAGACGGTGACCAGGAG.

Note: Primers for PCR2 of antibody light variable genes (Figure 2) (restriction enzyme sites are in shown with underline). To increase the cleavage efficiency, several base pairs (NNNNN) should be added on either side of the recognition site of restricted enzymes.

Odp 2571 (Forward-*EcoRI*): NNNNNGAATTCGCAGGCTGTGCTGACTCAG.

Odp 3677 (Reverse- *AvrII*): NNNNNCCTAGGACGACKGTCAGTGTGGTSCC.

60. Prepare 2% (wt/vol) agarose gel.

Remove PCR2 plate from the thermocycler and load 5 μL of PCR2 product of each well and 7 μL 1 kb plus ladder onto 2% agarose gel.

Note: Cover PCR2 plate and store the remaining PCR product at 4°C until purification and digestion.

61. Run the gel at 120 V for 30–45 min in 1× TAE buffer.
62. Expose the gel to UV light. The expected product for VH is ~350–500 bp and for VL is ~350–400 bp (Figure 3).

Pause point: Store PCR2 plate at −20°C or −80°C (stable for 3 years).

Note: Plate can be proceeded to the purification and digestion step immediately (without any need to be stored at −20°C).

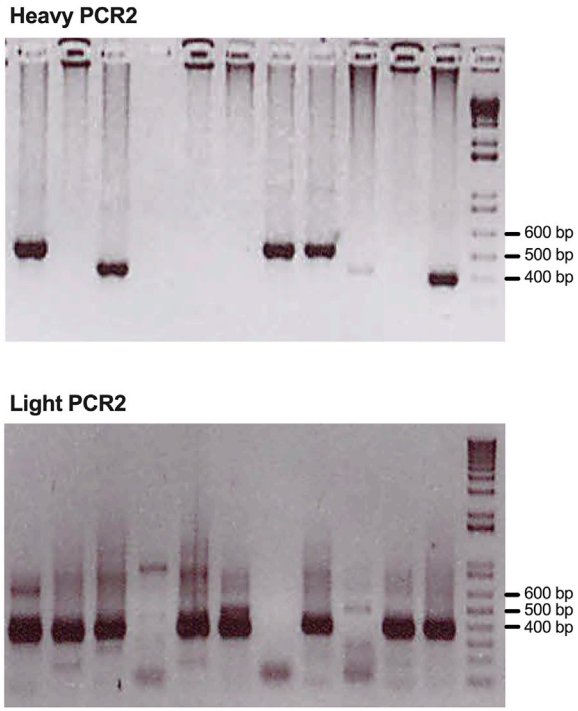

Figure 3. Gel electrophoresis of PCR products
PCR products of bovine heavy and light variable genes (PCR2) on 2% agarose gel with the size standard markers of defined base pair length (bp) indicated on the right.

Cloning of VH and VL genes into expression vectors

Timing: ~ 3–4 days (For steps 63 to 109)

This step describes step-by-step detail on cloning of bovine variable genes into human expression vectors which results in IgG1 chimeric bovine mAbs with human constant regions.

Purification and digestion of PCR2 product

63. Follow the protocol for NucleoSpin Gel and PCR Clean-up kit (Scientifix, #740609.250) to purify 45 µL of PCR2 product.

Note: elute PCR2 product in 20 µL Nuclease-free H_2O.

Pause point: If not using the purified PCR2 product immediately, store it at 4°C (stable for a few days) or −20°C and −80°C (stable for 3 years).

Note: In this protocol PCR2 is not sequenced. Sequencing is performed on heavy and light expression plasmids after confirming the antigen specificity of mAbs in ELISA.

64. Digest purified PCR2 product as below.

PCR product digestion mix

Reagent	VH	VL
CutSmart buffer	2.3 µL	2.3 µL
EcoRI-HF	0.25 µL (5 U)	0.25 µL (5 U)

(*Continued on next page*)

Continued		
Reagent	VH	VL
NheI-HF	0.25 μL (5 U)	–
AvrII	–	1 μL (5 U)
Purified PCR2 product	Up to 20 μL	Up to 20 μL

65. Incubate for 2 h at 37°C.
66. Follow the protocol for NucleoSpin Gel and PCR Clean-up kit (Scientifix, #740609.250).

Note: Elute digested PCR2 product in 20 μL Nuclease-free H_2O.

Pause point: If not using the PCR2 product immediately, store it at 4°C (stable for a few days) or −20°C (stable for a few month).

Linearization of expression vector

Expression vectors contain human heavy (IgG1) and light (lambda) constant regions.

67. Thaw IgG1 (pFUSEssCHIg-hG1, Invivogen) and lambda (pFUSE2ss-CLIg-hL2, Invivogen) expression vectors at 20°C–25°C.
68. Digest expression vectors as below.

Vector digestion mix		
Reagent	pFUSEssCHIg-hG1	pFUSE2ss-CLIg-hL2
CutSmart buffer	4 μL	4 μL
EcoRI-HF (20 U/μL)	0.5 μL (10 U)	0.5 μL (10 U)
NheI-HF (20 U/μL)	0.5 μL (10 U)	–
AvrII (5 U/μL)	–	2 μL (10 U)
Expression vector	10 μg	10 μg
Nuclease-free H_2O	Up to 40 μL	Up to 40 μL
Total	**40 μL**	**40 μL**

69. Incubate for 2 h at 37°C.
70. Add 5.5 μL Antarctic Phosphatase reaction buffer and 10 μL Antarctic Phosphatase.
71. Incubate for 1 h at 37°C.
72. Prepare 1% (wt/vol) agarose gel.
73. Load the whole volume of each digestion mix and 7 μL of 1 kb plus ladder into separate gel wells.
74. Run the gel at 100 V for 1 h in 1× TAE buffer.
75. Expose the gel to UV light and cut out the linearized vector with scalpel (Figure 4).

Note: The size of linearized pFUSE2ss-CLIg-hL2 vector is 3,837 bp and linearized pFUSEs-sCHIg-hG1 vector is 4,481 bp.

⚠ CRITICAL: Reduce the exposure time to UV light to minimise DNA damage and use new scalpel for each vector to prevent cross-contamination.

76. Follow the protocol for NucleoSpin Gel and PCR Clean-up kit (Scientifix, #740609.250). The expected yield of linearized vector after gel extraction and purification is 2–3 μg.

Note: Elute the digested linearized vector in 100–200 μL Nuclease-free H_2O.

Pause point: Make a few aliquots from the digested linearized vector and store them at −20°C (stable for 3 years). Avoid repeated freeze-thaw cycles.

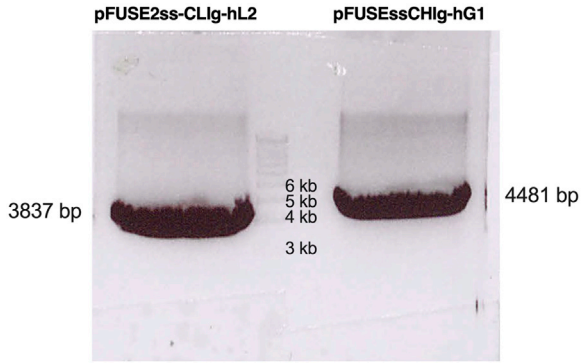

Figure 4. Linearization of expression vectors

pFUSE2ss-CLIg-hL2 (light) and pFUSEssCHIg-hG1 (heavy) expression vectors containing human antibody constant genes are digested and linearized with *EcoRI-HF/AvrII* and *EcoRI-HF/NheI-HF*, respectively. The linearized vectors are dephosphorylated and run on 1% gel to cut out a band of 3,837 bp and 4,481 bp for pFUSE2ss-CLIg-hL2 and pFUSEssCHIg-hG1, respectively.

77. Adjust the concentration of linearized vector to 50–100 ng/ μL with Nuclease-free H_2O.

Ligation of digested VH/VL genes & transformation

78. Remove the T4 DNA ligase buffer (Thermo Fisher Scientific) from −20°C and thaw it at 20°C–25°C.
79. Remove purified digested PCR2 product and digested purified linearized expression vectors from −20°C and thaw them on ice.

Note: Remove T4 DNA ligase (Thermo Fisher Scientific) from −20°C and keep it on ice, just immediately before adding it to the ligation mix.

80. Prepare the ligation mix for each individual PCR2 product.

Note: Ligation mix for heavy and light gene must be prepared in separate microtubes.

Note: A ligation control can must be included with vector only (no purified digested PCR2 product). This control verifies that the vector is digested completely and whether the phosphatase treatment worked properly.

Ligation mix	
Reagent	
Purified digested PCR2 product	10 μL
Purified digested linearized vector	1 μL (50–100 ng)
T4 DNA ligase	1 μL
T4 DNA ligase buffer	1.3 μL
Total	**13.3 μL**

81. Incubate the ligation reaction at 20°C–25°C for 12–18 h.
82. Remove Top10 E. Coli chemically competent cells from −80°C and thaw them on ice.
83. Add 40 μL Top10 competent cells to the ligation reaction and keep it on ice for 30 min.
84. Set the water bath to 42°C.
85. Incubate the ligation reaction at 42°C for 45–60 s and place it on ice immediately.
86. Incubate on ice for 5 min.

87. Add 100 μL of SOC medium to each reaction and incubate on a bacterial shaker at 37°C and 180 rpm for 1 h.
88. Meantime, label Zeocin agar plate for heavy chain reactions and Blacticidin LB-agar plate for light chain reactions. Prewarm the agar plate at 37°C.
89. After incubation (step 87), centrifuge the samples briefly to collect the evaporated medium.
90. Transfer the whole volume of each sample on the corresponding plate and spread the cells smoothly using bacterial spreader.
91. Incubate the plates at 37°C for 12–18 h.

Pause point: LB-agar plate can be sealed with parafilm and store at 4°C for up to 1 month after step.

Colony PCR and sequencing

Colony PCR is performed to verify the ligation of insert and sequencing confirm the accuracy of cloning.

92. Remove agar plates from the incubator or fridge.

⚠ CRITICAL: Vector only plate must have lower colonies compared to the sample plates.

93. Prepare 10 mL conical centrifuge tubes containing 100 μL Zeocin or Blacticidin LB-broth for heavy chain and light chain plates, respectively.
94. Prepare colony PCR mix, transfer 20 μL to each well of PCR 96-well plate, and keep it on ice.

Colony PCR mix	
Reagent	
GoTaq® Green Master Mix	10 μL
Forward primer	0.12 μL
Reverse primer	0.12 μL
Nuclease-free H_2O	9.76 μL
Total	**20 μL**

Note: Primers for colony PCR of heavy variable genes are Odp 3668 (Forward) Odp 2568 (Reverse). Primers for colony PCR of light variable genes are Odp 2571 (Forward) Odp 3677 (Reverse).

95. Clean pipette and bench surface with 70% ethanol.
96. With a sterile pipette tip, pick a single bacterial colony from the plate and transfer it to the colony PCR plate.
97. Eject the same tip to the corresponding 10 mL conical tube containing LB-broth Zeocin or Blacticidin.

Note: Pick 3 colonies from each plate and transfer them into separate colony PCR reactions and liquid culture.

98. Repeat this step for all agar plate.
99. Keep the liquid culture tubes at 20°C–25°C or 4°C until verifying the clones in colony PCR.
100. Centrifuge the colony PCR plate briefly, put it in the thermocycler and run colony PCR program.

Colony PCR cycling conditions			
Steps	Temperature	Time	Cycles
Initial denaturation	94°C	5 min	1
Denaturation	94°C	30 s	30 cycles
Annealing	50°C	30 s	
Extension	72°C	30 s	
Final extension	72°C	10 min	1
Hold	4°C	Forever	

101. Prepare 2% (wt/vol) agarose gel.
102. Remove Colony PCR plate from the thermocycler and load 5 μL of PCR product of each well and 7 μL 1 kb plus ladder onto 2% agarose gel.
103. Run the gel at 120 V for 30–45 min in 1× TAE buffer.
104. Expose the gel to UV light. The expected product for VH is ~350–500 bp and for VL is ~350.
105. For each pair of correct heavy and light chains, add 3 mL Zeocin LB-broth (heavy chain) or Blasticidin LB-broth (light chain) to the liquid cultures/
106. Incubate liquid culture tubes at 37°C for 12–18 h.
107. Follow the protocol for NucleoSpin Plasmid kit (Scientifix, #740588.250) to purify DNA plasmids 2 mL of liquid culture.

Note: Elute DNA plasmid in 50 μL Nuclease-free H2O.

Pause point: If not using DNA plasmid immediately, store it at −20°C (stable for 3 years).

108. Send DNA plasmid to sequencing using Odp 2781 sequencing primer (CTCAACTCTAC GTCTTTGTTTC) to confirm the accuracy of cloning.
109. Use IMGT/V-QUEST (http://www.imgt.org/IMGT_vquest/vquest)[5,6] or IGBLAST (https://www.ncbi.nlm.nih.gov/igblast/)[4] tools to analyze the heavy and lambda light chains.

Expression of bovine-human chimeric mAbs

Timing: 5–10 days (for steps 110 to 149)

This step described the details of chimeric mAbs expression and performing functional assays to confirm the activity of HIV-1 specific chimeric mAbs.

Small-scale transfection of Expi293F cells

Transfection of mAb plasmids can be conducted in 12-well (3.8 cm^2) to confirm the expression of mAb chains and to evaluate the function of chimeric mAb.

⚠ CRITICAL: Decontaminate the hood, bench surface and pipettes with 70% ethanol prior to use to avoid any possible contamination.

110. Use Expi293F cells (Thermo Fisher Scientific) and prepare transfection mix with Expifectamine 293 Transfection kit (Thermo Fisher Scientific, #A14525) in Opti-MEM (Gibco) media.

Note: Using Opti-MEM medium prevents cell culture from overgrowth.

Small-scale transfection (values per well)										
Plate	Light DNA	Heavy DNA	Media for DNA	No. of cells	Media for cells	Expifec*	Media for Expifec*	Enhancer 1	Enhancer 2	Total vol
12-well	1,200 ng	800 ng	100 μL	5×10^6	1.7 mL	5.5 μL	100 μL	10 μL	100 μL	2 mL

*Expifectamine.

111. Dilute 2 μg DNA plasmids (1,200 ng light chain DNA plasmid and 800 ng heavy chain DNA plasmid) in 100 μL prewarmed Opti-MEM. Incubate at 20°C–25°C for 5 min.
112. Dilute 5.5 μL Expifectamine in 100 μL prewarmed Opti-MEM.
113. Add diluted Expifectamine to diluted DNA and mix it.
114. Incubate the transfection mix at 20°C–25°C for 20–30 min.
115. Meantime, prepare 5×10^6 cells in 1.7 mL prewarmed Opti-MEM.

⚠ CRITICAL: Cell viability should be ≥95%.

116. Add the transfection mix to the cells slowly and swirl gently during addition.
117. Incubate the flask on an orbital shaker (100–130 rpm) with 8% CO_2 at 37°C for 18–22 h.
118. Add 10 μL enhancer 1 and 100 μL enhancer 2 to the cells and swirl gently.

Note: There is no need to prewarm enhance 1 and enhancer 2.

119. Harvest the supernatant 5 days post-transfection (4 days after adding enhancers).
120. Spin the harvested supernatant at 500 × *g* for 5 min and transfer the clear supernatant to a clean microtube to be tested in western blot and ELISA.

Pause point: If not testing the supernatant, it can be stored at 4°C (stable for a few weeks).

Note: Supernatant can be tested in western blot to confirm the expression of both heavy and light chain (Figure 5).

Functional assay

ELISA or functional assays can be performed to evaluate the activity of chimeric mAbs before large-scale production. The below protocol if for evaluation of anti-HIV chimeric mAbs.

121. Dilute D7324 Sheep anti-gp120 antibody (Aalto Bio Reagents) in DPBS to make the final concentration of 2 μg/mL.
122. Coat polystyrene microtiter ELISA plate with 100 μL of the diluted D7 324 Sheep anti-gp120 antibody and incubate the plate at 4°C for 12–18 h.

Note: Alternatively, plate can be coated with the diluted D7 324 Sheep anti-gp120 antibody and incubated at 37°C for 2 h.

Note: pH of DPBS as coating buffer must be 7.2–7.4.

123. Wash the plate 4 times with DPBST (DPBS containing 0.1% Tween-20) and 2 times DPBS.
124. Block the plate with 200 μL of 5% skim milk (in DPBST) for 1 h at 20°C–25°C. Then wash the plate as step 123.
125. Dilute D7324 tagged- AD8 SOSIP gp140. V4.1 in 5% skim milk to make the final concentration of 600 ng/mL and add 100 μL to each well.
126. Incubate the plate for 1 h at 20°C–25°C and afterwards wash the plate as step 123.
127. Add 100 μL from serial dilutions of harvested supernatant to the plate and incubate for 2 h at 20°C–25°C.

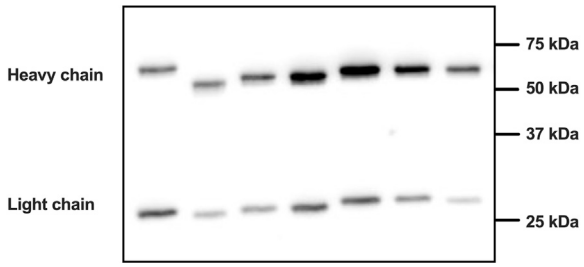

Figure 5. Heavy and light mAb chains on 12% SDS-PAGE reducing gel with apparent molecular weight marker protein positions annotated in Dalton units on the right

Note: Supernatant must be diluted with DPBS (at least 1:1 ratio) to reduce the background and false positive signal.

128. Meantime, prepare 1/1000 dilution of goat anti-human IgG HRP (KPL) and pre-incubate with 2% normal sheep serum for 2 h at 20°C–25°C.
129. Wash the plate as step 123 and add 100 µL of diluted goat anti-human IgG HRP.
130. Incubate the plate for 1 h at 20°C–25°C then wash the plate as step 123.
131. Add 100 µL of SureBlue TMB Peroxidase Substrate (KPL) and incubate the plate for 1 h at 20°C–25°C.
132. Stop the reaction using 100 µL of 2 M sulfuric acid.
133. Measure the absorbance at 450 nm against a reference of 690 nm using an ELISA plate reader.

Note: Sample is considered positive if optical density is at least 2 times greater than optical density of negative control.

Large-scale transfection of Expi293F cells

Transfection of mAb plasmids can be conducted in 125 mL Erlenmeyer flask with vent cap.

⚠ CRITICAL: Decontaminate the hood, bench surface and pipettes with 70% ethanol prior to use to avoid any possible contamination.

134. Prepare the transfection mix as below:

Note: Prepare Midiprep from the expression vectors by using NucleoBond Xtra Midi kit if they are not enough for transfection.

large-scale transfection

Flask	Light DNA	Heavy DNA	Media for DNA	No. of cells	Media for cells	Expifec*	Media for Expifec*	Enhancer 1	Enhancer 2	Total vol
125 mL	15 µg	10 µg	1.5 mL	75×10^6	25 mL	80 µL	1.4 mL	150 µL	1.5 mL	30 mL

*Expifectamine.

135. Dilute 25 µg DNA plasmids (15 µg light chain DNA plasmid and 10 µg heavy chain DNA plasmid) in 1.5 mL prewarmed Opti-MEM. Incubate at 20°C–25°C for 5 min.
136. Dilute 80 µL Expifectamine in 1.4 mL prewarmed Opti-MEM.
137. Add diluted Expifectamine to diluted DNA and mix it.
138. Incubate the transfection mix at 20°C–25°C for 20–30 min.
139. Meantime, prepare 75×10^6 cells in 25 mL prewarmed Opti-MEM.

⚠ CRITICAL: Cell viability should be ≥95%.

140. Add the transfection mix to the cells slowly and swirl the culture flask gently during addition.
141. Incubate the flask on an orbital shaker (100–130 rpm) with 8% CO_2 at 37°C for 18–22 h.
142. Add 150 μL enhancer 1, and 1.5 mL enhancer 2 to the flask and swirl the flask gently.

Note: There is no need to prewarm enhance 1 and enhancer 2.

143. Harvest the supernatant 5 days post-transfection (4 days after adding enhancers).
144. Spin the harvested supernatant at 500 × *g* for 5 min and transfer the clear supernatant to a clean microtube.
145. Again, spin the supernatant at 3,000–4,000 × *g* for 10–15 min to remove the debris.
146. Transfer the supernatant into a sterile 50 mL conical tube.

Pause point: The supernatant can be kept at 4°C (several days). It is recommended to add Sodium Azide (0.02%–0.05%) to prevent bacterial growth if the supernatant is stored for a longer time.

Purification of chimeric mAb

147. Equilibrate poly-prep chromatography columns and add 1 mL Protein G agarose (50% slurry) to the column and purify the mAbs according to the manufacturer's instructions (https://www.thermofisher.com/order/catalog/product/20398).
148. Elute the mAbs with 10 mL Glycine (50 mM, pH: 2.7) and neutralize low pH with 300 μL Tris (1 M, pH: 8.5).

Note: It is recommended to do buffer exchange with DPBS 1× using 50K Amicon centrifugal filters (Merck).

149. The chimeric mAb productivity can be determined by measurement with nanodrop. The presence of heavy and light chains is confirmed in western blot and quantified using ImageJ.[7]

Pause point: The purified mAbs (in DPBS buffer) can be stored at −80°C for a couple of years.

EXPECTED OUTCOMES

In Flow cytometry, debris and aggregated cells are eliminated by and SSC-A/W and FSC-A/H, respectively. Then live IgG AF488+ cells are selected. Antigen-specific bovine B cells (PE+/APC+) are gated and single sorted into 96-well plate. Staining of cells from non-vaccinated cows shows the background level and helps to set the accurate gating.

Following cDNA synthesis and nested RT-PCR, the amplified heavy and light variable genes are detected by gel electrophoresis. Heavy variable genes that are bigger than 400 bp are ultralong CDRH3 mAbs.

The analysis of mAb genes can provide information about the mAb clonal families. Antibodies that share the same heavy and light germline genes belong to one clonal family. It is recommended to analyse the mutations in nucleotide sequence of V genes compared with the corresponding germline V genes. Nucleotide insertion and deletion in VDJ junction make it impossible to determine the mutation in D and J genes.

In transfection into Expi293 cells, the mAbs are produced with the productive and natural pair of heavy and light chain genes. Western blot confirms the expression of both heavy and light chains whereas ELISA assay can test the function of mAbs.

LIMITATIONS

There is no commercial anti-bovine memory B cells marker available at the time of publishing this protocol, thus only IgG+ cells are sorted. This results in a significant number of sorted cells (1–3 full 96-well plates) which is due to sorting of all the cells binding to anti-bovine IgG (not only memory-B cells).

The efficiency of antigen-specific single cell sorting is 30%–50% in FACS which mean more than half of antigen-specific bovine cells are lost during the process. To overcome this limitation, the FACS operator must be careful about gating and settings of single cell sorting.

TROUBLESHOOTING

Problem 1

Cell clumps before sorting (step 41).

Potential solution

Filter stained cells before sorting and add more buffer to dilute the cells if required.

Problem 2

The percentage of antigen specific cells is low (step 42).

Potential solution

Check the serum. The cow may have low antigen-specific mAb titre and poor vaccine response. Blood collection time is also critical. Five days after a booster vaccination is when memory B-cells are likely to be in peripheral circulation. Dead cells can also bind to the antigen and reduce the chance of antigen capture by live cells. Remove dead cells with Ficoll-Pauqe before staining PBMCs.

Problem 3

Negative control in nested PCR yields positive band or all the wells turns positive with identical DNA band size (step 62).

Potential solution

It is possible to have contamination in PCR mix, pipette, pipette tips or bench surface. Clean the surface and keep the surface and equipment nucleic-acid free. Make small aliquots from Nuclease-free water to avoid contamination. Do not work with mAb expression plasmids using the same pipette or in the same area when planning to perform nested PCR.

Problem 4

There are very few or no band after nested PCR (step 62).

Potential solution

RNAs from single cells may have been degraded. Do not store sorted single cells for a long time. Avoid freeze-thaw and make cDNA from single cells as soon as possible.

Problem 5

Upon transformation of ligation control (containing only expression plasmid without any insert), a lot of colonies are obtained (step 91).

Potential solution

Expression vector has not been linearized adequately or vector has been re-ligated due to incomplete dephophorylation. Make sure to digest and dephophorylate the expression vector completely (increase the digestion and dephophorylation incubation time if required).

Problem 6
Upon transformation of ligation products, no colony is obtained (step 91).

Potential solution
Make sure that insert and vector purification is efficient or increase the insert to vector ratio.

Problem 7
mAb is not expressed (step 120).

Potential solution
Make sure that there is no stop codon in the sequence of heavy and light chain genes. The sequences can be out-of-frame. Check the cells for contamination.

Problem 8
mAb is not binding to the antigen (step 133).

Potential solution
Confirm that both heavy and light chains are expressed (by western blot). If the chains are expressed, the affinity of mAb is low. Use lower antigen concentration in FACS single cell sorting to isolate only high affinity mAbs.

Problem 9
The concentration of purified mAb is low (step 149).

Potential solution
Run transfection media from which the mAb has been purified in western blot. If the mAb bands are not present, the expression of mAb have been low as the cells may not be confluent enough or passaged for too long. It is possible that the mAb is naturally expressed at low level. Transfect more flasks if required. If the mAb bands are present in western blot, protein G beads have reached the capacity and use fresh beads.

Problem 10
The elution of mAb is low (step 149).

Potential solution
Need to optimise the pH of Glycine. Alternatively, can use 50 mM citrate with pH of 2.6 or lower.

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Prof. Damian Purcell (dfjp@unimelb.edu.au).

Materials availability
This study did not generate new unique reagents. All materials are available commercially.

Data and code availability
This study did not generate any unique datasets or code.

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AUTHOR CONTRIBUTIONS

B.H. developed and wrote the protocol. D.F.J.P. critically read and revised this protocol.

DECLARATION OF INTERESTS

B.H. and D.F.J.P. are inventors on a corresponding patent from the University of Melbourne: HIV-1 antibodies PCT/AU2021/050593.

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